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Original Article

Effect of heavy metals in the meconium on preterm mortality: Preliminary study

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Abstract ***Background***: There have been many studies that have investigated the risk factors of mortality in preterm infants, but none has shown an association between preterm mortality and exposure to heavy metals or trace elements. The aim of this study was therefore to measure the levels of toxic metals (lead, cadmium) and trace elements (zinc, iron, copper) in meconium samples and elucidate their association with preterm mortality.

Methods: Metals and trace elements were measured in the meconium of 304 preterm infants using a flame atomic absorption spectrophotometer.

Results: The level of heavy metals and trace elements in non-surviving infants was significantly higher than in surviving infants. Moreover, the level of heavy metals and trace elements in non-surviving infants whose gestational age was <30 weeks ($n = 11$) was significantly higher than in surviving infants ($n = 12$). Receiver operating characteristic curve analysis showed that gestational age and meconium lead level predicted early mortality in premature newborns. Furthermore, this curve analysis showed that, when comparing meconium lead level and gestational age, meconium lead level had a similar effect on mortality as gestational age.

Conclusion: Meconium lead level and gestational age are associated with increased mortality risk in preterm neonates.

Key words cadmium, lead, mortality, premature birth.

Prematurity is the leading cause of neonatal morbidity and mortality.[1] The mortality of premature infants has decreased in Turkey over the past decades, but Turkey still has a higher neonatal mortality than in other developed countries.[2,3] Perinatal mortality is an indicator of the health of the mother and child and may reflect the conditions of reproductive health, which are related to socioeconomic status, quality of antenatal care, and delivery factors. Some of the risk factors identified for prematurity or mortality are related to the mother and complications that occur during pregnancy, labor, and delivery, which may affect both the fetus and the newborn. These include maternal hypertension, placenta praevia, premature placental detachment, other abnormalities of the placenta, respiratory distress syndrome, cardiovascular disorders specific to the perinatal period (such as patent ductus arteriosus), infections, intrauterine growth restriction and low birthweight.[4,5] More recently, studies have indicated that exposure to air pollution may be associated with low birthweight and preterm birth.[6] In addition, urban air pollution has also been directly implicated in perinatal mortality.[7] The association between death in the neonatal period (0–28 days of life) and air pollution was identified by Lipfert *et al.* and Hajat *et al.*[8,9] To our knowledge, however, there have been no studies that demonstrate an association between preterm mortality and heavy metals or trace elements in newborn meconium. The newborn intensive care unit (NICU) at Kocaeli University is located in west Anatolia, an industrial city that contains 17% of all businesses and factories in Turkey. Turkey's largest shipyard and tire factory are in Anatolia. In addition, there are numerous industrial factories, including petroleum, plastic, rubber, dye, chemicals, farming, drugs, iron, steel, copper, automobile spare parts, cable, glass, lime, and ceramic factories. These factories are stationed alongside the highway between Istanbul and Kocaeli. Moreover, Anatolia is located along major highways, the autobahn, and rail connecting Europe and Asia.

The objectives of this study were to measure the levels of heavy metals (lead and cadmium) and trace elements (zinc, iron and copper) in meconium samples and elucidate their association with early mortality (during the first 48 h of life) in preterm newborns.

Methods

This study was approved by the Kocaeli University Medicine Faculty Ethics Committee (Ethics Committee number: 108/15/20). Premature infants who were hospitalized in the NICU were included in the study group. Infants with congenital abnormalities were excluded. Interviews in person with all the mothers were performed at the hospital after delivery. The interviews investigated the potential confounding factors, demographic

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factors before and after pregnancy and risk factors for mortality (fetal distress, multiple births, pre-eclampsia/eclampsia, maternal disease, drug and cigarette use during pregnancy, infection, short birth intervals, premature rupture of membranes, polyhydramnios, and oligohydramnios); this information was completed in a form for each infant.

The last menstrual period and the new Ballard score were used to determine gestational age. A history of urinary tract infection and vaginitis was obtained from the mother to diagnose maternal infections. In addition, acute phase reactants (white cell count and C-reactive protein level) and the use of antibiotics were recorded from the maternal records. If the interval between the current gestation and a prior gestation was <2 years, close delivery was assumed. General infant characteristics, including gender, gestational age, birthweight, type of birth, and Apgar scores, as well as demographic features of the parents (age, education, social security status, occupation, income, and number of people living in the same house), were recorded. Birthweight percentiles were evaluated according to Battaglia and Lubchenco.[10] Infants whose birthweights were under the 10th percentile were identified as small for gestational age.

The education level of the parents was categorized into six groups: (i) illiterate; (ii) literate; (iii) primary school (grades 1–5); (iv) secondary school (grades 6–8); (v) high school (grades 9–11); and (vi) university. The social security status of the parents was recorded as follows: (i) no security; (ii) health security for the poor, called a 'green card' in Turkey; (iii) social insurance institution, a type of health security for private firms and workers; (iv) pension fund, health security for government employees; (v) social security for artisans and the self-employed; and (vi) private insurance.

In 2007, the Turkish Statistical Institute declared the monthly poverty level for a family of four to be 619 Turkish liras. In this study, the parental economic status was classified into two categories: low income level (<155 Turkish liras/month individual income); and middle or high income level. Parents were grouped according to socioeconomic status by a Barratt score (Barratt simplified measure of social status). The sums of the average education level points and occupation points of the parents were used for the Barratt score.

Meconium analysis

Meconium samples, which were collected with a wooden pipe from the diaper, were put into non-metal cups, quickly frozen and kept at −20°C. Meconium samples were collected from preterm babies who were alive before trace elements or iron intake. Meconium levels of lead, cadmium, zinc, copper and iron were determined using a flame atomic absorption spectrophotometer (Shimadzu AA-680) at the biophysics laboratory of Cerrahpasa Medical Faculty, as previously described.[11] The minimum detectable limits for lead, cadmium, zinc, copper and iron were 0.045, 0.009, 0.037, 0.080, and 0.014 μg/mL, respectively. The range of recovery of trace elements (zinc, copper and iron) in meconium was 92.4–96.7%, 93–101%, and 92–98%, respectively, and the percent variance was approximately 2.6% (2.2–3.1%). The recovery of heavy metals (lead and cadmium) in meconium ranged between 100% and 102%. The inter-assay coefficient of variability ranged between 0.34% and 8.9%; the intra-assay coefficient of variability ranged between 0.44% and 8.44%. The precision, measured as coefficient of variance, was between 3.9% and 6.7% for cadmium.[11,12] The metal levels were standardized by the dry weight of the sample in grams and then divided by the baby's weight in kg.

Statistical analysis

For many variables in the collected data, the standard deviations were wide, and the distributions were not normal. Consequently, statistical analysis was performed by calculating the median and minimum/maximum. Differences between groups were analyzed using the Mann–Whitney *U*-test, the chi-squared test and the Fisher's exact test. Receiver operating characteristic (ROC) curve analysis was used to predict early mortality of premature newborns.

Results

All infants were hospitalized in the NICU. Prenatal risk factors and demographic characteristics are listed in Table 1. The surviving group ($n = 291$) and the non-surviving group ($n = 13$) had statistically similar distributions of gender and Barratt scores and similar incidences of multiple births, pre-eclampsia, maternal infection, close delivery and premature rupture of membranes. Apgar score at 1 and 5 min, gestational age and birthweight in non-surviving infants were significantly lower than those in surviving infants (Mann–Whitney *U*-test, χ^2 test; Table 1). The rate of respiratory distress syndrome in non-surviving infants was significantly higher than in surviving infants (χ^2 test; Table 1).

Heavy metals and trace elements in non-surviving infants were significantly higher than in surviving infants (Mann–Whitney *U*-test; Table 2). Heavy metals and trace elements in non-surviving infants whose gestational age was <30 weeks ($n = 11$) were significantly higher than in surviving infants ($n = 12$; Mann–Whitney *U*-test; Table 3). ROC curve analysis was used to predict early mortality in premature newborns.

The ROC curve analysis showed that gestational age and meconium lead level predicted early mortality in premature newborns (Fig. 1). Moreover, when comparing meconium lead level and gestational age, the meconium lead level had a similar effect on mortality as gestational age (Fig. 1).

Discussion

To our knowledge, this is the first study to evaluate the association between early neonatal mortality in preterm infants and the exposure of pregnant women and their newborn babies to heavy metals (lead and cadmium) and trace elements (copper, iron and zinc).

In this study, analysis focused on the effects of heavy metals and trace mineral exposure on birthweight, mediated by reduced fetal growth, as opposed to early delivery. Therefore, the subject group was restricted to infants who were born at gestational age <37 weeks. In this prospective study, we found several important clinical factors that were associated with mortality in premature infants. Gestational age, birthweight, and Apgar scores at 1 and

Table 1 Prenatal risk factors and demographic characteristics

	Surviving ($n = 291$) *n* or median (min–max)	Non-surviving ($n = 13$) *n* or median (min–max)	*P*
Gestational age (weeks)			
24–26	3	6	
27–29	9	5	
30–32	65	0	
33–36	214	2	
Birthweight (g)	2070 (700–3820)	715 (570–2510)	<0.001
Small gestational age	10	3	>0.05‡
Barratt score	20 (3–63)	19 (5–63)	>0.05†
Sex (F/M)	137/154	6/7	>0.05‡
Prenatal risk factors			
Multiples	96	2	>0.05‡
Singletons	198	8	>0.05‡
Pre-eclampsia	51	1	>0.05‡
Maternal infection	33	1	>0.05‡
Close delivery	15	1	>0.05‡
Premature rupture of membranes	68	4	>0.05‡
Placenta praevia	16	0	>0.05‡
Gynecologic problem	23	0	>0.05‡
Cervical insufficiency	7	0	
Maternal chronic disease	24	1	>0.05‡
Maternal smoking	12	1	>0.05‡
Natal risk factors			
Type of birth cesarean	245	7	>0.05‡
Apgar (1 min)	8 (0–9)	4 (3–9)	**<0.001**†
Apgar (5 min)	9 (4–10)	4 (4–10)	**<0.001**†
Postnatal risk factors			
Respiratory distress syndrome	77	11	**0.001**‡
Sepsis or pneumonia	22	1	>0.05‡

†Mann–Whitney *U*-test. ‡χ^2 or Fisher's exact test.

5 min were associated with mortality in the present study, as shown in previous studies.[13–15] Lead and cadmium levels in meconium were also associated with early mortality. We assessed the important risk factors for early neonatal death, including Barratt score, maternal reproductive history, prenatal risk factors, type of birth, maternal hypertension, placenta previa, premature placental detachment, other abnormalities of the placenta and sepsis.[4] These risk factors in surviving preterm newborns were similar to those in non-surviving preterm newborns. The incidence of respiratory distress syndrome, intrauterine growth restriction and low birthweight in non-surviving preterm newborns was significantly higher compared to that in surviving preterm newborns.

Table 2 Meconium toxic metals and trace element levels vs survival

	Surviving ($n = 291$) Median (min–max)	Non-surviving ($n = 13$) Median (min–max)	*P*
Lead (ng/g/kg)	14.9 (5.2–57.1)	44.1 (14.2–73.8)	**<0.001**
Cadmium (ng/g/kg)	1.2 (0.36–5)	3.5 (1–7.9)	**<0.001**
Zinc (ng/g/kg)	92 (29–361)	259 (61–498)	**<0.001**
Iron (ng/g/kg)	55.6 (19.9–227)	153.3 (48.8–201)	**<0.001**
Copper (ng/g/kg)	48.2 (18.4–167)	155 (37–179)	**<0.001**

Mann–Whitney *U*-test.

Table 3 Heavy metals and trace elements: Gestational age <30 weeks

	Surviving ($n = 12$) Median (min–max)	Non-surviving ($n = 11$) Median (min–max)	*P*
Lead (ng/g/kg)	30 (20.1–47.1)	43.8 (20–73.8)	**0.001**
Cadmium (ng/g/kg)	3.4 (1.7–6.3)	3.5 (0.8–7.9)	>0.005
Zinc (ng/g/kg)	235 (163–328)	226 (126–498)	>0.005
Iron (ng/g/kg)	127 (75–2026)	146 (66–201)	>0.005
Copper (ng/g/kg)	116 (69–167)	131 (53–179)	>0.005

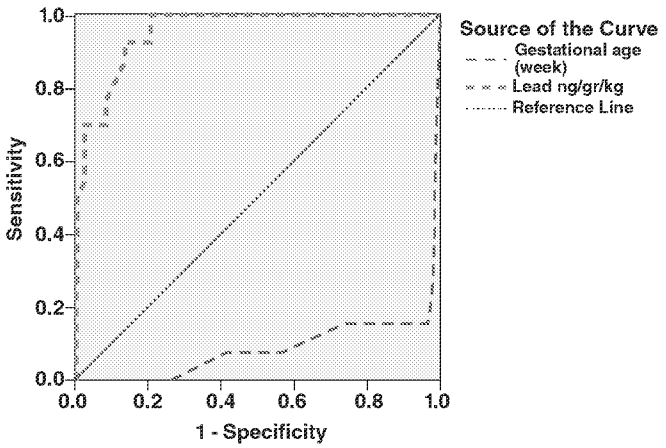

Fig. 1 Receiver-operating characteristic curve analysis for premature newborn mortality. Gestational age and lead as predictors of mortality. Gestational age has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased. (– – –) Gestational age (weeks); (• • •) lead (ng/g per kg); (- - -) reference line.

On bivariate analysis, the meconium metals level differed between surviving and non-surviving newborns. When newborns with gestational age <30 weeks were compared, only meconium lead level differed between groups. Non-surviving newborns in this group had a significantly higher meconium lead level compared to surviving newborns. Moreover, ROC curve analysis showed that, when comparing meconium lead level and gestational age, the meconium lead level had a similar effect on mortality as gestational age (Fig. 1).

Nevertheless, we cannot exclude residual confounding effects by other factors for which we did not analyze, such as mercury or arsenic level. Background air pollution is correlated with temperature and/or other meteorological parameters, and its effect on health might vary with the season. Therefore, we elected to study meconium because heavy metals and trace elements accumulate in the meconium during gestation. Moreover, the presence of heavy metals and levels of trace elements in fetal blood may not accurately reflect the degree of fetal exposure to heavy metals or the excessive or deficient levels of trace elements. Meconium is a substance that can be obtained easily and non-invasively and is representative of a long duration of fetal exposure to pesticides or metals during gestation.[12,15–20] Most studies have investigated the association between deaths in the perinatal period and air pollution.[8,9,21,22] To our knowledge, however, no study has focused on the association between lead, cadmium, copper, iron or zinc levels in meconium and mortality. Few studies have investigated the effect of blood heavy metals on prematurity or growth.[23,24] It has been suggested that in dry meconium, concentration of trace elements >100 μg/g (0.01%) can indicate toxicity.[25] Furthermore, meconium samples should not contain toxic metals, such as lead and cadmium, in normal conditions. Therefore, we suggested that the trace levels of these elements in the meconium were toxic.

Lead may directly injure developing neurons and astrocytes and may also produce alterations in neurotransmission and cell signaling. Health problems in children that are related to environmental contaminants may follow from developmental disturbances during the intra-uterine period or during the first years of life. Prenatal lead exposure is a major risk factor for impaired fetal and infant development; during the early embryonic and fetal stages, lead can pass through the placenta and affect the nervous system.[26,27] The association between lung cancer in adulthood and cadmium was also identified in a review article written by Schoeters *et al.*[28] They noted that although transfer to the neonate through the placenta and through breast milk is limited, teratogenic and developmental effects were observed in experimental animals. In school-aged children, urinary cadmium level has been associated with immune-suppressive effects.[28] These studies suggest that fetuses and newborns may suffer from the consequences of a contaminated environment.

To our knowledge, there have been no studies that show an association between preterm mortality and heavy metals or trace elements in newborn meconium. This study supports previous conclusions regarding the negative effects of lead on mortality. More studies, however, are needed to confirm these results. There are several limitations to this preliminary study, including the small sample size of non-surviving premature newborns and the established difference in the median gestational age between the surviving and non-surviving groups. It is difficult to adjust for gestational age in non-surviving newborns because extremely premature deliveries are more common in non-surviving newborns. Therefore, it is likely that this study underestimated the differences between surviving and non-surviving newborns.

Conclusion

Meconium lead level and gestational age are associated with increased mortality risk in preterm neonates.

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